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Cadophora malorum and *Cryptosporiopsis ericae* isolated from medicinal plants of the *Orchidaceae* in China

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Abstract —Two species in the anamorphic genera *Cadophora* and *Cryptosporiopsis* are newly recorded as endophytes from medicinal plants of the *Orchidaceae* in China. *Cadophora malorum* was isolated from a stem of *Bletilla striata* in Hubei Province, and *Cryptosporiopsis ericae* from a root of *Spiranthes sinensis* in Tibet. These are the first records of these fungi from plants of the *Orchidaceae*.

Key words — endophytic fungi, taxonomy

Introduction

Orchids are unique among plants in their modes of nutrition (myco-heterotrophy) involving direct and often obligate relationships with fungi (Leake 1994). Thus, fungi are critical for an orchid's growth and development. Orchid mycorrhizas have been historically regarded as the third distinct structural lineage of mycorrhizas in addition to ecto-related and arbuscular mycorrhizas (Imhof 2009). Recently, non-mycorrhizal endophytic fungi associated with orchids have been shown to serve as potential growth promoters and source of bioactivity substances (Guo & Wang 2001), implying further application in the fields of cultivation and natural medicine.

During a survey of endophytic fungi associated with traditional medicinal plants of *Bletilla striata* (Thunb.) Rchb.f. and *Spiranthes sinensis* (Pers.) Ames (*Orchidaceae*) in China, *Cadophora malorum* and *Cryptosporiopsis ericae* were isolated from plant tissues. These are the first records of these anamorphic species from orchids.

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Materials and methods

Eighty-eight strains of endophytic fungi were isolated from healthy orchid plants of *Bletilla striata,* collected from Lichuan County, Hubei Province, and fifty-five strains from *Spiranthes sinensis*, collected from Linzhi County, Tibet. The isolation of endophytic fungi was performed by the modified method described by Bayman et al. (1997). In brief, roots and stems were surface-sterilized in a sequence of 75% ethanol for 1 min, 2.5% NaClO for 5 min, 75% ethanol for 1 min, and then rinsed in sterile distilled water. The endophytic fungi were first identified morphologically from published descriptions and the identifications confirmed through sequence analyses. After the extraction of genomic DNA from pure fungal cultures, the ITS regions were amplified and sequenced. Sequences were compared with fungal ITS sequences in GenBank using BLAST searches. These isolates are preserved as living cultures in the China General Microbiological Culture Collection Center, Institute of Microbiology, Chinese Academy of Sciences (CGMCC).

Taxonomy

Cadophora malorum (Kidd & Beaumont) W. Gams, Studies in Mycology 45: 188, 2000. Fig. 1 A–B

Colonies on PDA after 2 weeks in the dark at room temperature 2.0 cm diam, brown, usually with white margin. Mycelium superficial and immersed. Aerial mycelium bristly, composed of pale brown, smooth thick hyphae. Colony margin irregularly wavy. Conidiospores simple, straight or slight flexuous, hyaline and smooth, monophialidic phialides, integrated and terminal or discrete, ampulliform, lageniform with hyaline collarettes. Conidia simple, straight, oblong, rounded at the ends, colorless, smooth, 2–3 × 0.3–0.5 μm (Fig. 1A–B).

Specimens examined: **CHINA**: HUBEI PROVINCE, Lichuan County, in *Bletilla striata* (*Orchidaceae*) stem, 10 Sept. 2004, Zhi-Xia Meng BJ-10-1(CGMCC10118)

Remarks: *Cadophora* has been treated as a synonym of *Phialophora* (Conant 1937). Gams (2000) suggested using the generic name *Cadophora* for *Phialophora*-like species with affinities to the *Dermateaceae* in the *Helotiales*. Harrington & McNew (2003) molecular analyses supported Gams' view that members of the genus *Cadophora* were anamorphs of the *Helotiales* and distinguished from the morphologically similar anamorphic genus *Phialophora* in the *Chaetothyriales. Cadophora* species differ from true *Phialophora* species by pale to hyaline collarettes on top of their phialides (Gams 2000). In fact, morphological identification of the two genera was difficult because pigmentation in these species is often quite variable (Harrington & McNew 2003), making it necessary to combine morphological and molecular observations to identify

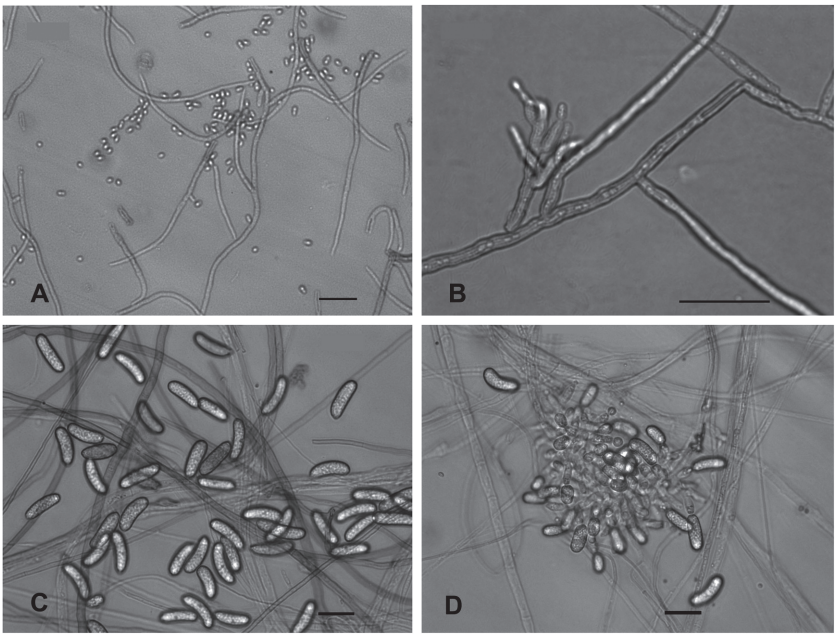

Fig. 1 *Cadophora malorum* (A–B) (CGMCC10118) and *Cryptosporiopsis ericae* (C–D) (CGMCC10119) showing conidia and phialides. Scale bar = 20μm

them. Morphology and ITS sequence (FJ450054) of our sample are identical to *C. malorum* (100% similarity with GenBank sequence DQ404350 from *Cadophora malorum*).

The known *Cadophora* species appear to be plant pathogens, root associates, or wood colonizers (Harrington & McNew 2003). *Cadophora malorum* is a common species in the genus that has been identified as a plant pathogen (Frisullo 2002). In our study, *C. malorum* was isolated from a stem of healthy *Bletilla striata*. The exact relationship between *C. malorum* and the orchid host plant needs further study.

Cryptosporiopsis ericae Sigler, Studies in Mycology 53: 57, 2005. Fig. 1 C–D

Colonies on PDA at room temperature after 21 d up to 8.0 cm diam, flat, felty, white to gray at the beginning and becoming grayish orange with age. Pale yellowish brown droplets occurred in the centre. Colony reverse gray orange when pigments produced. Conidiospores phialides, formed in hemispherical sporodochial conidiomata. Hyphae of young conidiomata moniliform and hyaline, older conidiomata composed of yellowish or black brown hyphae. Conidial masses white initially, becoming to pale to golden yellow in age.

Macroconidia cylindrical, slightly curved, rounded at the apex, nonseptate, smooth, hyaline, becoming to golden yellow and guttulate in age, 18–23 × 5.5–7.8 µm (Fig. 1 C–D). Microconidia nonseptate, hyaline, oblong, 10–12 × 4–5.5 µm (not shown).

Specimens examined: CHINA: TIBET, in root *Spiranthes sinensis* (*Orchidaceae*), Aug. 2007, Zhi-Xia Meng SC-b-2 (CGMCC10119).

Remarks: *Cryptosporiopsis ericae* was isolated and described from ericaceous plant roots from western North America (Sigler et al. 2005). Characteristics of conidiomata and conidia of our specimen coincided with the original description. Moreover, the ITS sequence of Chinese material (GU945547) was 99% identical to the *C. ericae* sequence (AY853167) in the GenBank database.

Many *Cryptosporiopsis* species are known from roots of woody plants, especially from ericaceous plants (Kowalski & Bartnik 1995, Verkley et al. 2003). The Chinese record is the first report of the species from herbaceous orchid plant root.

Although some species of *Cryptosporiopsis* (e.g. *C. radicicola*, a frequent colonizer of oak roots) may be host specific, the precise ecological roles in host roots remain unknown (Kowalski & Bartink 1995). *Cryptosporiopsis ericae* has been isolated from ericaceous roots, but Berch et al. (2002) found no formation of mycorrhizal structures (hyphal coils) in re-synthesis experiments done with salal (*Gaultheria shallon*) and *C. ericae*. Similarly, Wang et al. (2007) indicated that *C. ericae* was endophytic but non-mycorrhizal and non-pathogenic for their inoculated host, *Populus tremuloides* Michx.

In addition, cryptocandin (a unique lipopeptide antimycotic) has been described from *Cryptosporiopsis* sp. that might be useful clinically for the treatment of a variety of mycoses (Fischer et al. 1984, Strobel et al. 1999). The role of *C. ericae* in the medicinal host plant needs to be studied to establish whether it is associated with pharmacodynamic effects.

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Literature cited

Bayman PL, Lebrón RL, Tremblay JL. 1997. Variation in endophytic fungi from roots and leaves of *Lepanthes* (*Orchidaceae*). New Phytologist 135: 143–149.

Berch SM, Allen TR, Berbee ML. 2002. Molecular detection, community structure and phylogeny of ericoid mycorrhizal fungi. Plant and Soil 244: 55–66.

Conant NF. 1937. The occurrence of a human pathogenic fungus as a saprophyte in nature. Mycologia 29: 597–598.

Fischer PJ, Anson AE, Petrini O. 1984. Novel antibiotic activity of an endophytic *Cryptosporiopsis* sp. isolated from *Vaccinium myrtillus*. Transactions of the British Mycological Society 83: 145–148.

Frisullo S. 2002. First report of *Cadophora malorum* on *Asparagus officinalis* in Italy. Phytopathologia Mediterranea 41(2): 148–151.

Gams W. 2000. *Phialophora* and some similar morphologically little-differentiated anamorphs of divergent ascomycetes. Studies in Mycology 45: 187–199.

Guo SX, Wang QY. 2001. Character and action of good strain on stimulating seed germination of *Gastrodia elata*. Mycosystema 20(3): 408–412.

Harrington TC, McNew DL. 2003. Phylogenetic analysis places the phialophora-like anamorph genus *Cadophora* in the *Helotiales*. Mycotaxon 87: 141–151.

Imhof S. 2009. Arbuscular, ecto-related, orchid mycorrhizas—three independent structural lineages towards mycoheterotrophy: implications for classification? Mycorrhiza 19: 357–363.

Kowalski T, Bartnik C. 1995. *Cryptosporiopsis radicicola* sp. nov. from roots of *Quercus robur*. Mycological Research 99: 663–666.

Leake JR. 1994. The biology of myco-heterotrophic (saprophytic) plants. *New Phytologist* **127**: 171–216.

Sigler L, Allan T, Lim SR, Berch S, Berbee M. 2005. Two new *Cryptosporiopsis* species from roots of ericaceous hosts in western North America. Studies in Mycology 53: 53–62.

Strobel GA, Miller RV, Martinez-Miller C, Condron MM, Teplow DB, Hess WM. 1999. *Cryptocandin*, a potent antimycotic from the endophytic fungus *Cryptosporiopsis* cf. *quercina*. Microbiology 145: 1919–1926.

Verkley GJM, Zijlstra JD, Summerbell RC, Berendse F. 2003. Phylogeny and taxonomy of root-inhabiting *Cryptosporiopsis* species, and *C. rhizophila* sp. nov., a fungus inhabiting roots of several Ericaceae. Mycological Research 107: 689–698.

Wang W, Tsuneda A, Fe Gibas C, Currah RS. 2007. *Cryptosporiopsis* species isolated from the roots of aspen in central Alberta: identification, morphology, and interactions with the host, in vitro. Canadian Journal of Botany 85 (12): 1214–1226.